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Effects of *Hedyotis leschenaultiana* DC (Rubiaceae) Whole Plant Extract on Sperm Parameters and Sex Hormones in Adult Male Rats

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Abstract
Fertility enhancement effect of ethanol extract of whole plant of *Hedyotis leschenaultiana* was observed in male albino rats. The relative weight of the testis and epididymis were increased. The epididymal sperm count, motility and sperm abnormality were increased significantly (P<0.01) in treated rats. There was an increase in serum protein, albumin, globulin, urea, creatinine and the activity of liver marker enzymes (SGOT, SGPT and ALP) levels of treated rats. The activities of serum antioxidants (CAT, SOD. GPX, GST and GRD) in whole plant extract treated rats were increased. The results of the hormonal assay showed that increased serum levels of LH and testosterone but decreased in the serum levels of estrogen compared to control. The results of fertility test indicated that the treated adult male rats increased the number of female's impregnation. In addition, the number of implantations and the number of viable fetuses were also increased. The results of the present study concluded that, ethanol extract of whole plant of *Hedyotis leschenaultiana* enhanced sperm concentration, motility and testosterone which might produce positive result in the male fertility enhancement.
Keywords: *Hedyotis leschenaultiana*, testosterone, fertility, FSH

INTRODUCTION

Throughout the ages, men and women have incessantly pursued all means to enhance, maintain and bring back their sexual ability, or to stimulate the sexual desire for the opposite sex. One of the most recurrent methods has been the use of aphrodisiacs. Herbal medicines are a major source of aphrodisiacs and have been used, wordwide for thousands of years by different cultures and civilizations to enhance sexual performance. Natural herbs that are popularly considered to have properties like enhancing potency, enhancing sexual functioning or improving sexual performance make up large segment of current day herbal markets. On a broader scale, the herbal aphrodisiacs may also include products that have adaptogenic, tonic, revitalizing and rejuvenating properties. The natural supplements in this class are called herbal aphrodisiacs. Traditional herbs represent an extra ordinary reservoir of active ingredients which are still present in about 25% of all prescriptions of modern medicines[1, 2].

Plants as an important source of medicines and play a key role in the health of the world's population. The use of plant materials to treat sexual disorders have a long history in most countries, and plant materials have been proven effective in improving sexual desire and sexual behaviour in male animals[3,4].

The genus *Hedyotis* finds a prominent place in different Indian systems of medicine. The different ethnic communities in India have used different species of *Hedyotis* in the treatment of various ailments[5] *H.leschenaultiana* whole plant were reported for the biological activities such as *in vitro* antioxidants activity, Oral glucose tolerance test and hepatoprotective activity[6-8]. Taking into consideration for the medicinal importance of *H.leschenaultiana*, the ethanol extract of whole plant of *H.leschenaultiana* was designed to investigate the fertility enhancement effect in adult male rat.

MATERIALS AND METHODS

Plant Materials

The whole plant of *Hedyotis leschenaultiana* DC was collected from Kothagiri, Nilogiri Biosphere Reserve, Western Ghats, Tamilnadu. The plant were identified with the help of local flora, voucher specimen were preserved in the Ethnopharmacology unit, Research department of Botany, V.O.Chidamabaram College, Tuticorin, Tamilnadu.

Preparation of plant extract

The whole plant of *H.leschenaultiana* were dried separately under shade and then powdered with a mechanical grinder to obtain a coarse powder, which were then subjected to extraction in a Soxhlet apparatus using ethanol. The ethanol extract was concentrated in a rotatory evaporator. The concentrated ethanol extract of whole plant of *H.leschenaultiana* were used for fertility enhancement activity.

Animals

Normal healthy male Wistar albino rats (180-240g) were used for the present investigation. Animals were housed under standard environmental conditions at temperature (25±20C) and light and Dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats of either sex selected by random sampling were used for acute toxicity study[9]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric

incubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 upto 2000 mg/kg body weight.

EXPERIMENTAL DESIGN

The male rats were divided into 4 groups consisting of 5 animals.

Group I: Rats received normal saline daily for 14 days, orally. (Normal control).

Group II: Rats received ethanol extract of whole plant of *H.leschenaultiana* at the dose of 100mg/kg body weight daily for 14 days. Group III: Rats received ethanol extract of whole plant of *H.leschenaultiana*, at the dose of 200mg/kg body weight daily for 14 days.

Group IV: Rats received ethanol extract of whole plant of *H.leschenaultiana*, at the dose of 300mg/kg body weight daily for 14 days.

After 24 hours of last treatment, the final weight was recorded and the animals were sacrificed by decapitation. Blood was collected. Sera were separated by centrifugation at 3000 rpm for 10 minutes and stored at 20^{o}C until used for various biochemical assays. Then testis, epididymis, vas deferens, seminal vesicle and ventral prostate were dissected out, trimmed off extraneous and weighed accurately on torsion balance. The organs weights were expressed in terms of mg/100g body weight.

Sperm count

Epididymal fluid (for sperm count) was collected from caput and caudal segments separately and diluted with Sorenson's buffer (pH7.2). The separated fluid was taken for sperm count. Sperm count was carried out by using Neubauer's haemocytometer as described by Zaneveld and Pelakoski[10].

Sperm motility and abnormality

After anaesthetizing the rats, the caudal epididymis was then dissected. An incision (about 1mm) was made in the caudal epididymis and drops of sperm fluid were squeezed onto the microscope slide and 2 drops of normal saline were added to mobilize the sperm cells. Epididymal sperm motility was then assessed by calculating motile spermatozoa per unit area.

Morphology (abnormality) was evaluated on sperm from the caudal epididymis. The total morphological abnormalities were observed as described by Linde et al[11].

Serum biochemical analysis

Serum proteins[12] and serum albumins were determined by quantitative colorimetic method by using bromocresol green.

The total protein minus albumin gives the globulin, urea[13], creatinine[14], serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by using the method of Reitman and Frankel[15]. Serum alkaline phosphatase (ALP) was measured by the method of king and Armstrong[16].

Serum antioxidants Serum antioxidant

Catalase (CAT)[17], Superoxide dismutase (SOD)[18], Glutathione peroxidase (GPX)[19], Glutathione s-transferase (GST)[20] and Glutathione reductase (GRD)[21] were analyzed.

Hormonal Assay

Blood removed from the rats by intracardiac method. Blood was centrifuged at 3000 rpm to separate the serum for the measurement of testosterone, Luteinizing hormone (LH), estrogen and follicle stimulating hormone (FSH). The quantitative determination of hormones was done by using Enzyme Immunoassay Method (EIA). The EIA kit was obtained from Immunometrics (London, UK).

Fertility Test

Fertility was estimated in adult male rats treated with ethanol extracts of whole plant of *H.leschenaultiana* and in the control male counterparts. Each male was placed in an individual cage with two virgin untreated females of the same strain. They were left together for 10 days during which two estron cycles had elapsed. One week after the removal of the exposed males, pregnant females were killed by cervical dislocation under light ether anesthesia and the number of implantation sites, the number of fetuses and the number of resorption sites were recorded[22].

Statistical Analysis

Data were expressed as Mean ± SEM. Student's t test was used for statistical comparison.

Results

FERTILITY ENHANCEMENT

Body and reproductive organ weight

The administration of ethanol extract of whole plant of *H. leschenaultiana* to rats did not cause any significant change in the body weight (Table 1) and on the libido of treated rats; whereas, weights of testis and other accessory sex organs were increased significantly ($p < 0.05$) (Table 1). Among the accessory sex organs, a significant weight gain was seen in the caput and caudal epididymal segment. Slight decrease was observed in vas deferens (VD) seminal vesicle (SV) and prostate.

Sperm count and sperm motility

Sperm motility and sperm density in caudal epididymis, significantly increased (Table 2) and the increase was severe in whole plant extract of *H. leschenaultiana* (Group IV) followed by whole plant extract of *H. leschenaultiana* (Group III) and the same trend was seen in the caput epididymal sperm density when compared to control (Group I).

Sperm abnormality

Sperm abnormality in caput and caudal region was not affected by ethanol extracts of *H. leschenaultiana* whole plant and ($p < 0.05$) (Table 2). Among the studied concentrations, 300 mg/kg concentration of extract of *H. leschenaultiana* have shown significant and improved the sperm morphology.

Serum antioxidants

The activities of CAT, SOD, GPx, GST and GRD in the serum of control and whole plant extract treated rats were presented in Table 3. In the present study, plant extract treated rats had shown increased activities of all the studied antioxidants when compared to control rat (Table 3).

Table 1: Effect of *H. leschenaultiana* whole plant extract on the Body and Reproductive organ weight of adult male albino rats

Treatment Groups	Body wt(gm)		Testis (gm)	Epididymis (mg)		VD (mg)	SV (mg)	Prostate (mg)
	Before	After		Caput	Cauda			
Group-I	195.35±6.21	211.61±5.72	2.064±0.11	232.16±3.93	294.52±5.67	123.11±3.93	269.32±5.33	214.13±5.16
Group-II	186.24±3.56	199.54±5.56	1.993±0.34	204.21±2.83	281.19±5.08	134.29±4.55^{ns}	286.15±4.04^{ns}	223.45±6.54^{ns}
Group-III	216.34±5.92	229.63±5.14*	2.534±0.28**	248.71±3.94**	324.59±6.23*	143.15±5.04*	291.69±5.84*	232.66±5.93*
Group-IV	204.19±3.83	238.51±4.32**	2.611±0.74**	244.94±4.31**	346.11±5.89**	149.63±4.88*	304.55±6.81**	246.14±4.84**

Each Value is SEM of 5 animals * $p < 0.05$; ** $p < 0.01$. Control vs Treated ns : not significant

Table 2: Effect of *H. leschenaultiana* whole plant on the sperm concentration and motility in the epididymis of adult male albino rats

Treatment Groups	Sperm Concentration (Counts x 10^6 mil)		Sperm Motility (FMI) @ (cauda)	Sperm Abnormality #	
	caput	cauda		Head (%)	Tail (%)
Group-I	293.63±6.91	349.54±8.39	168.51±7.55	5.23±0.11	9.14±0.84
Group-II	311.49±8.37ns	369.14±10.81ns	179.50±8.14ns	4.61±0.14	7.05±0.33
Group-III	324.11±9.23	398.56±8.94**↑	193.10±9.14**↑	4.83±0.24	7.84±0.74
Group-IV	339.63±10.66	454.16±9.36**↑	198.63±8.55**↑	4.03±0.26	5.88±0.16*↓

Each Value is SEM of 5 animals * $p < 0.05$, ** P<0.01 Control vs Treated:
↓-Significantly reduced; ↑ significantly increased
@: Motility is movement recorded after 5 min in suspension of caudal epididymal spermatozoa in phosphate buffered solution.
#: Expressed in percentage

Table 3: Effect of *H. leschenaultiana* whole plant extract on the activity of serum Catalase, Glutathione peroxidase, Glutathione-S transferase, and Superoxide Dismutase and Glutathione reductase in adult albino rats

Parameter	Treatment Groups				
	Group I	Group II	Group III	Group IV	Units
Catalase	8.98±0.35	9.22±0.65^{ns}	12.84±0.34*↑	14.09±0.63**↑	µ moles of H_2O_2 decomposed/min/mg protein
Glutathione peroxidase	0.422±0.01	0.471±0.05^{ns}	0.532±0.04**↑	0.651±0.03**↑	µ moles of NADPH oxidized/min/mg protein
Glutathione-Stransferase,	11.54±0.96	10.12±0.54^{ns}	11.05±0.37^{ns}	14.63±1.22*↑	µ moles of conjugate formed/
Superoxide Dismutase	21.62±0.78	18.34±0.79	24.96±0.45*↑	26.05±0.67**↑	Units
Glutathione reductase	18.67±0.45	20.66±0.84^{ns}	22.06±0.42^{ns}	26.87±0.34*↑	µ moles of NADPH

Each Value is SEM of 5 animals * $p < 0.05$, ** p<0.01 Control vs Treated: ↑ significantly increased.

Serum biochemical profile

Serum protein, albumin, globulin, urea and creatinine and the activity of liver marker enzymes (SGOT, SGPT and ALP) levels of control and whole plant extract treated rats were depicted in Table 4. No significant changes were noted in the serum biochemical and liver marker enzymes in the entire drug treated groups when compared to control group.

REPRODUCTIVE HORMONE PROFILE

Serum testosterone level

The ethanol extract of whole plant of *H. leschenaultiana* (100, 200 and 300 mg/kg body weight) repeated treatment daily for 14 days caused a significant increase in the serum level of testosterone in male rats (Table 5).

Serum luteinizing hormone (LH) level

Repeated treatment of male rats with the *H. leschenaultiana* extracts for 14 days caused a dose related increase in the serum level of LH (Table 5). The level of increase was statistically significant (Group IV) ($p < 0.05$).

Serum estrogen level

The ethanol extract of *H. leschenaultiana* (100, 200 and 300 mg / kg body weight) caused a significant decrease in the serum level of estrogen in male rats. Dose of 300 mg / kg body weight administered daily for 14 days caused a sharp decline in the serum level of estrogen (Table 5).

Serum follicle stimulating hormone (FSH) level

Pretreatment with ethanol extract of whole plant of *H. leschenaultiana* for 14 days caused an increase in the serum level of FSH in male rats compared to control (Table 5). The increase in the serum level of FSH in male rats was statistically significant when treated with *H. leschenaultiana* ($p < 0.05$) respectively.

Table 4: Effect of *H. leschenaultiana* whole plant extract on the serum biochemical profile of adult male albino rats

Parameter	Treatment Groups				
	Group I	Group II	Group III	Group IV	Units
Protein	7.58±0.14	8.14±0.26	8.55±0.14	8.86±0.32*↑	gm/dl
Albumin	4.50±0.11	4.94±0.09	5.04±0.26	4.96±0.17	gm/dl
Globulin	3.08±0.23	3.20±0.14	3.51±0.17	3.40±0.21	gm/dl
Urea	12.63±0.21	15.27±0.16	18.36±0.34	14.33±0.15	mg/dl
Creatinine	0.71±0.09	0.82±0.05	0.69±0.10	0.73±0.12	mg/dl
SGOT (U/L)	14.36±0.84	19.16±0.56	18.33±0.69	13.16±0.19	(U/L)
SGPT(U/L)	19.33±0.84	21.22±0.84	19.74±0.74	20.11±0.56	(U/L)
ALP(U/L)	174.39±4.51	182.65±4.64	198.66±5.11	189.16±5.21	(U/L)

Each Value is SEM of 5 animals * $p < 0.05$ – Control vs Treated: ↑ significantly increased

Table 5: Effect of *H. leschenaultiana* whole plant extract on the Sex hormone levels in male albino rats

Treatment Groups	Parameters			
	Testosterone (ng/ml)	LH (µIu/ml)	Estrogen(pg/ml)	FSH (µIu/ml)
Group I	3.94±0.13	3.65±0.16	17.27±0.98	1.08±0.03
Group II	4.03±0.22	5.92±0.15ns	15.37±0.63ns	1.84±0.07
Group III	4.54±0.56*	7.84±0.23*	10.56±0.71*	2.63±0.05*
Group IV	4.93±0.16*	8.44±0.67*	11.32±0.54*	2.95±0.07*

Each Value is SEM of 5 animals * $p < 0.05$, ** $p<0.01$ Control vs Treated: ↓ significantly reduced; ↑ significantly increased.

Table 6: Effect of *H. leschenaultiana* whole plant extract on the Fertility of adult male albino rats

Groups	No. of Male	No. of Females	No. of Pregnant Females	No. of Implantation	No. of Viable fetuses	Total No. of Resorption Sites
Group-I	3	10	7/10 (70%)	8.83±0.27	6.50±0.56	4
Group-II	3	10	8/10 (80%)	9.15±0.24	7.50±0.53	4
Group-III	3	10	10/10 (100%)**↑	11.27±0.16	8.25±0.21*↑	5
Group-IV	3	10	10/10 (100%)**↑	11.84±0.16*↑	9.15±0.45*↑	5

Each Value is SEM of 5 animals * $p < 0.05$, ** $p<0.01$ Control vs Treated; ↑ significantly increased.

FERTILITY TEST

The results presented in Table 6 shows that intragastric administration of extracts of whole plant of *H. leschenaultiana* (100, 200 and 300 mg/Kg body weight) for 14 days to male rats causes a significant decrease ($p< 0.05$) in the number of females impregnated by male treated rats. The number of viable fetuses calculated after cesarean sections were significantly increased ($p< 0.05$) in female rats impregnated by treated males when compared with female impregnated with untreated male rats. On other hand, the number of resorption sites was found to be increased in female impregnated by treated male rats when compared to controls.

DISCUSSION

In the present study, the weight of reproductive organs markedly increased. The weight and secretary functions of testes, epididymis, seminal vesicles, ventral prostate and vas deference are closely regulated by androgens. The drug may act on pituitary gland and increased main hormone of spermatogenesis. It is well established fact that weights and secretary functions of the epididymis, seminal vesicle and ventral prostate are closely regulated by the androgens, changes taking place in these organs after castration can be counteracted by administration of testicular hormones thus serving as "indicator test" for the male hormones[23, 24].

The results presented in this work also show that the seminal vesicles weights were increased in adult male rats ingested *H. leschenaultiana*. This increase in the accessory glands weights might suggest an increase in the pattern of testosterone secretion. Significant increase in the sperm motility of caudal epididymis was observed in treated group. This may be due to activity effects of *H. leschenaultiana* on the enzymes of oxidative phosphorylation.

Sexual cells can occur during the reproductive phase, mitotic division of the spermatogenesis or during the maturation of the spermatozoa, thereby increasing the number and quality of the sperm cells produced in the testes. Among the ethanol extract of *H. leschenaultiana* whole plant (Group-II and III) (200 and 300 mg/kg body weight) produced a significant increase in total sperm count and viable sperms. This may be as a result of the ability of the extract at the given doses, to either interfere with spermatogenetic process in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadodropins which may result in alteration of spermatogenesis. The presence of mature sperm concentration was increased in the experimental rats treated with 300 mg/kg body weight *H. leschenaultiana* whole plant extract. This suggests that the 300 mg/kg dose could influence the maturation of the spermatozoa in the male

rats, which might also be a contributory factor to the increase in the mean total sperm count.

Similar to our results, Turk *et al*[25], Bin-Seng *et al*[26] have also shown that pomegranate juice and fractions of quassinoid – rich *Eurycoma longifolia* extract consumption significantly increased epididymal sperm concentration and motility and decreased abnormal sperm rate when compared to that of the control.

In the present investigation the observed increase in the caudal epididymal sperm motility might be due to an alteration in the microenvironment in the caudal epididymis, which also had a synergistic action on the of the spermatozoa of the treated rats as a result of the androgen-stimulatory effect of the extract of *H. leschenaultiana* whole plant. The increase in the caudal epididymis sperm counts in the treated animals substantiates the spermatogenic nature of the extract. The extract had a direct effect on the testes resulting in an increase in the number of spermatozoa and the increased level of testosterone production. Also, the extract had no spermatotoxic effect as previously indicated by Shah *et al*.[27].

Increase in LH concentration observed in the treated rats with 300mg/Kg of *H.leschenaultiana* whole plant extract with confer the increase in testosterone concentration. This may be an indication that the *H. leschenaultiana* has stimulatory effect on the hypothalamic – pituitary axis of the male rats[28].

The extract did not show an antigonadotrophic nature, demonstrated by the increased levels of FSH and LH in the treated rats. The increased level of FSH reveals a possible role of *H. leschenaultiana* whole plant extract in influencing the release of gonadotrophic hormones from the pituitary. The rise of FSH by itself is of critical importance in the initiation and expansion of spermatogenesis in mammals, as is generally agreed by Lohiya *et al*.[29].

The increased level of superoxide dismutase, catalase, glutathione peroxidase, glutathione s-tranferase and glutathione reductase were reported in the present study. Similarly, total protein, SGOT, SGPT and ALP levels were increased in the serum of extract treated rats.

The results presented in this paper also showed that, the ingestion *H.leschenaultiana* by adult male rats increased the number of impregnated females. The number of implantations and the number of viable fetuses were increased. This effect may be due to increase in sperm motility and sperm density.

The observed improvement potency of *H. leschenaultiana* may be due to presence of steroids, flavonoids and saponins which may increase sexual behaviour and have a positive effect on the sperm quality[30,31]. In other words, they stimulate the endogenous testosterone levels possibly by raising level of LH [28]. In the present study administration of *H. leschenaultiana* whole plant significantly increased the testosterone and LH concentrations without marked augment of FSH when compared to that of the control group.In conclusion, these results confirmed that, long term *H. leschenaultiana* ingestion produces increased effects on fertility on reproductive system in adult male rat. However, the exact mode of action requires further studies.

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